Keeping Our Drinking Water Safe Using Faster Cutting Edge Technology

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Purpose

Over the last three decades, the Centers for Disease Control and Prevention (CDC) and the US EPA have collected and reported data relating to occurrences and causes of waterborne-disease outbreaks in the United States. From 2000 through 2002, 13 states reported 17 outbreaks associated with drinking water and 10 of these outbreaks were attributed to parasitic and bacterial contamination. A number of these microorganisms are now listed on the 2003 Contaminant Candidate List (CCL) because of the need for more exposure research. In addition, the National Research Council (NRC) subcommittee has suggested that virulence factor activity relationships (VFARs) may provide a more rapid means of identifying waterborne pathogens than the current process that relies on exposure and health effects as the two primary categories for screening potential microbial drinking water contaminants. The purpose of this research project is to use state-of-the-art mass spectrometric techniques, such as electrospray ionization tandem mass spectrometry (ESI-MS/MS) and matrix-assisted laser desorption/ ionization-mass spectrometry (MALDI- MS), to identify virulence factors that enable the CCL microorganisms to cause disease. The goal of this research is to use this proteomic information to develop more sensitive and precise methods in order to gather occurrence data that will be used to create better EPA regulations for protecting humans from microbiological contaminants in U.S. drinking water supplies.

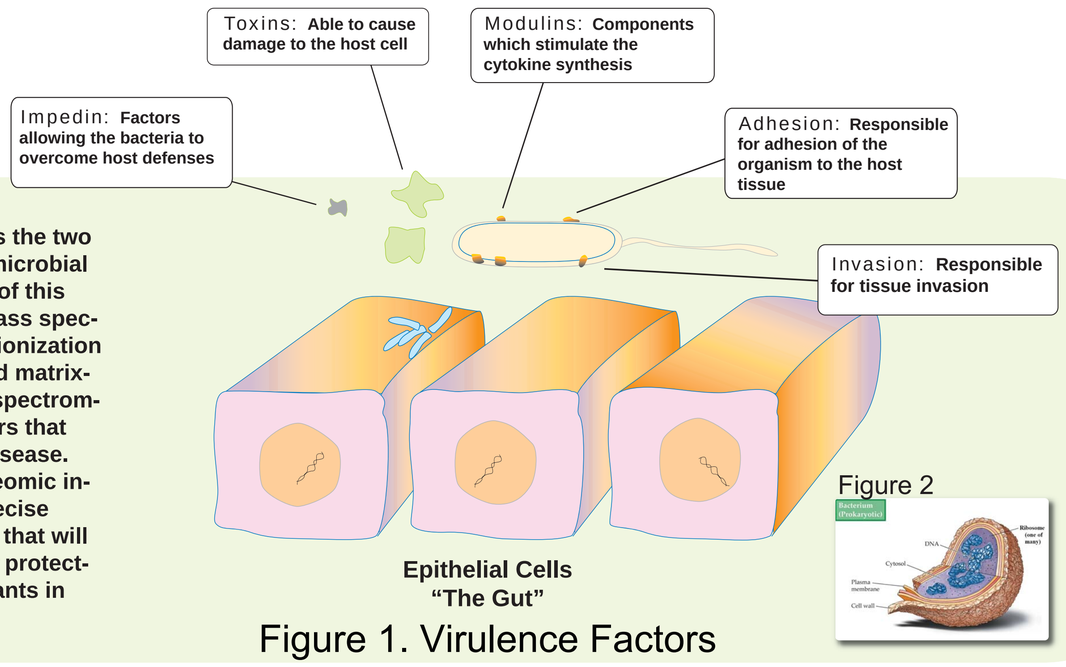


Figure 1. Virulence Factors

Figure 2

Approach

Virulence factors are identified using a multidiscipline approach. Classical protein isolation and purification techniques are used to isolate the desired targets. Biological assays are used to help identify the protein of interest based on a specific biological function (i.e., adhesion, toxicity, or cellular modification). Next, the isolated protein (that has a biological function) will be identified either by peptide mass fingerprinting (products of a protein digest) or by sequencing the amino acid chain for each peptide using either MALDI-MS or ESI-MS/MS. The spectrum achieved for each type of analysis will be compared against the National Center for Biotechnology Information (NCBI) protein databases to determined the identity of the protein.

Table 1. The sample preparation and biological assay will differ depending on which class of virulence factors is being studied:

Virulence Factor	Location	Biological Assay
Adhesion	Supernatant and cell membrane	Adhesion assays
Toxins (Exotoxins)	Supernatant	Cell toxicity
Invasion	Cytosol	Cell based assay
Impedins	Supernatant	*ROS and RNS
Modulins	Cytosol and cell membrane	*TNF analysis

* ROS - Reactive Oxygen Species, RNS - Reactive Nitrogen Species, and TNF - Tumor Necrosis Factor

Both MALDI-MS and ESI-MS/MS have been used increasingly over the past decade as proteomic tools to provide crucial protein sequencing information. This sequencing information can be used to determine the "identity" of the protein, if it is known, or to determine best "homology" by mining the sequence information against large protein sequence databases.

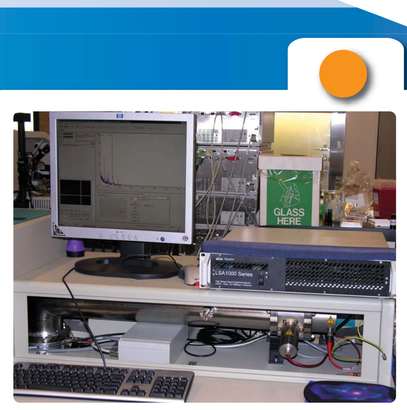


Figure 3: MALDI-MS

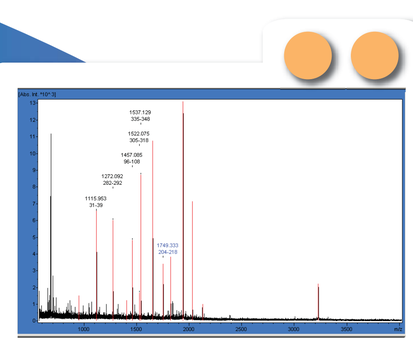


Figure 4: MALDI-MS Spectra
MALDI-MS spectra of the protein digest of VS-V51.

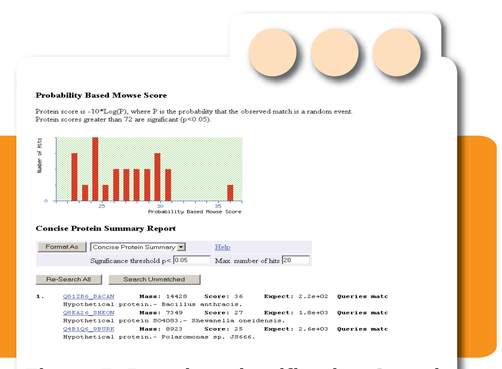


Figure 5: Protein Indentification Search Engine Results. Data mining results from query (mass values observed in spectra).

Figure 6: ESI-MS/MS

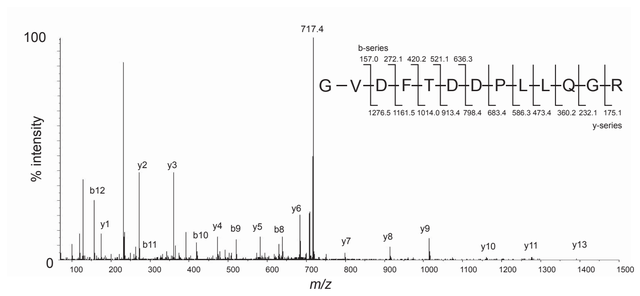


Figure 7: ESI-MS/MS Spectra
ESI-MS/MS spectra of a peptide amino acid sequence.

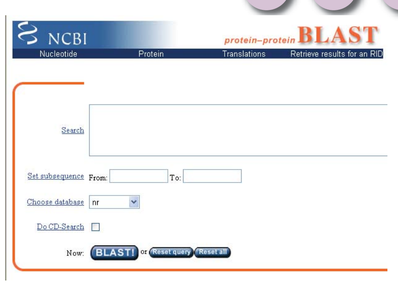
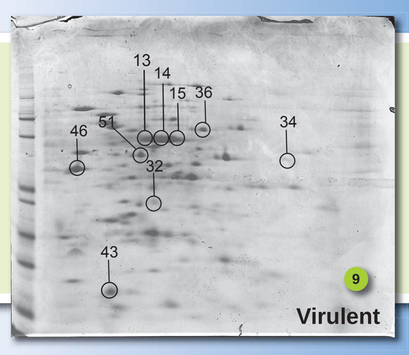
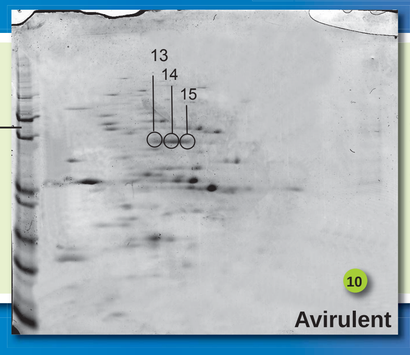


Figure 8: Search Engine
NCBI BLAST search engine (Protein short, nearly exact matches database).



Proteins in the virulent strain and NOT in the virulent strain
VS-V32: In progress
VS-V36: In progress
VS-V43: In progress
VS-V46: HlyA (Adhesion Protein)
VS-V51: In progress



Proteins in common between the virulent and avirulent strains.
Exotoxins
VS-S13: ACT (known Virulence Factor)
VS-S14, S15: Aerolysin (known Virulent Factor)

Figure 9 & 10
2D gels of supernatant (Exotoxins and Adhesion Factors) taken from A.) Virulent and B.) Avirulent strain of *Aeromonas*.

Virulent proteins of the CCL microorganism ***Aeromonas*** are currently being identified by comparing the protein expression of a virulent strain against an avirulent strain, using 2D gel electrophoresis. Note: The virulent and the avirulent strains were chosen based on infectious dosage (I.D.) information.

Status

- Current work is focused on identifying virulence factors for the CCL microorganism Aeromonas hydrophila.
- A virulent and avirulent pair of strains has been identified for two of the pathogenic species of *Aeromonas* (*A. hydrophila*, and *A. veronii bv sobria*).
- 2D gels of the virulent and avirulent strain of *A. veronii bv sobria* have been done (Figure 9 & 10).
- There are 12 proteins that are expressed by the virulent strain and not by the avirulent strain.
- Work is currently in progress to identify these proteins.
- Thus far, an adhesion protein and a few unique enzymes have been identified.

- Up-coming research will focus on the microorganisms *Legionella* and *Mycobacterium*.